IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Arjun SINGH	Group Art Unit: 1632
Application No.: 08/448,946	Examiner: S. Priebe
Filed: May 24, 1995	Confirmation No.: 1239
For: USE OF ALPHA FACTOR SEQUENCES IN YEAST EXPRESSION SYSTEMS	

DECLARATION OF RONALD A. HITZEMAN UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The undersigned, Ronald A. Hitzeman, does hereby declare and state that:

1) I make the following declaration based upon my knowledge and belief.

2) I received my Ph.D. in Biological Chemistry from the University of Michigan in 1977. Since that time, I have worked continuously on heterologous gene expression and secretion of heterologous (foreign to yeast) proteins by yeast. I have 38 publications in prestigious scientific books and journals and have 9 issued U.S. patents, as well as corresponding foreign patents which are listed on my curriculum vitae, attached.

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3) I directed the research at Genentech involving the isolation of the alpha factor gene (also independently isolated by Kurjan and Herskowitz) as well as a second alpha factor gene that they did not isolate.

4) My present employment is as a consultant for companies with yeast heterologous gene and heterologous protein secretion problems independently and through Genotypes, Inc. of which I am the president.

5) I have read and understand U.S. Patent No. 4,546,082 by Kurjan and Herskowitz from the Unversity of California in Berkeley, CA.

6) I agree with the following statements made by Anthony J. Brake in a declaration under 37 C.F.R. 1.132 signed 7/23/86 in U.S. Application Serial No. 06/487,950, filed April 25, 1983.

7) In my professional opinion, the Kurjan et al. working examples 9a, 9b and 9c are not enabling, and would not teach one of ordinary skill in the art how to successfully accomplish fusion of the alpha-factor gene with a gene coding for a precursor of somatostatin, with a gene for corticotropin, or with a gene for a precursor-enkephalin, nor would the description in these examples, if followed, provide results which would prove the utility of the yeast alpha-factor signal sequence for producing secreted mature heterologous proteins. The technical basis for my opinion is the following discussion of examples 9a, 9b and 9c, pointing out

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defects which prevent these examples from being capable of achieving their stated purpose.

A. Defects in Example 9a, Kurjan et al.

(1) Treatment of the Kurjan et al. RH1 fragment to fill-in the HindIII cohesive ends (presumably by T4 DNA polymerase or Klenow enzyme) would result in filling-in both the HindIII and EcoRI ends, thus destroying the EcoRI site by not allowing it to be reformed by hybridization to a complimentary sticky end. Kurjan et al., Example 9a at column 10, line 68 to column 11, line 5 says:

> "The cohesive end of the HindIII site of this fragment is filled in enzymatically to produce a fragment denoted RH2 to be ligated to a segment of the somatostatin gene. The RH2 fragment is jointed to a PstI-EcoRI fragment (denoted PE) from the sequence that codes for presomatostatin (Goodman et al.)"

This step permanently destroys this EcoRI site at the same time as it fills in the HindIII site on the R1-2 fragment.

(2) The proposed fusion to the preprosomatostatin cDNA is made at a PstI site which has been modified by poly(dC), poly(dG) homopolymers, the exact length of which was not determined (Goodman et al., P.N.A.S. (1981) 77:5869). Thus, there is only a 1/3 chance of the resulting presomatostatin fusion being in-frame for translation of the correct amino acid sequence. In addition, the polyG sequence and the three 5' nucleotides (AAG) will result in additional residues [$(Gly)_n$-Lys] (where n is polyG nucleotides/3, GGG codes for Gly, AAG codes for Lys) between the alpha-factor leader and preprosomatostatin. Such residues can interfere with secretion of

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a fusion protein, particularly when additional charged amino acids, such as lysine, are present.

(3) Blunt-end ligation of the fragments provides no way of ensuring the proper orientation in the ligation products and the fragments will be able to form linear and circular concatamers from which the desired products cannot be released by EcoRI digestion (since the EcoRI sites were destroyed by the fill-in reaction in (1) above). Twelve different orientations are equally possible for the blunt end ligation taking two DNA segments at a time. When larger DNA ligation chains form the number of possible orientations becomes extremely large. The blocked EcoRI site means that it cannot be used to liberate the desired products in order to screen for the correct sequence in order to clone the correct sequence and ensure production of the correct product.

(4) The PstI ends are 3' overhangs and thus cannot be filled in because of the specificity of T4 DNA polymerase or Klenow enzyme. The PstI ends must be rendered blunt by S1 nuclease digestion or by 3' exonuclease activity of T4 DNA polymerase or Klenow enzyme thus removing nucleotides. This is not discussed nor recognized by Kurjan et al.

(5) There are three PstI sites internal to the cDNA sequence, and thus a partial PstI digest must be performed to generate the desired PE fragment. A complete digestion which is presumed from the statement in Kurjan et al., column 11, lines 2-8, would destroy the desired "PE" DNA segment.

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(6) Cleavage in the prosomatostatin sequence is at Arg-105, not Lys-105 as stated at line 28, column 11.

B. Defects in Example 9b by Kurjan et al.

(1) The same problems in using the blunt-end RH2 fragment to fuse to the SS fragment will occur as in example 9a, thereby creating a mixture of different species of product with additional amino acids at the amino terminal end.

(2) The 3' SmaI site in the SS fragment ends in the middle of an Arg codon, therefore there will not be a proper translational termination signal in the fusion protein resulting in translational read-through. Translational read-through of this gene fusion results in the synthesis of a longer protein with an unknown C-terminal extension creating a fusion-protein other than ACTH alone. The extra amino acids might be removed by proteolytic processing as proposed, however it is likely that the additional amino acids would also interfere with proper secretion of the fusion protein and if not removed could destroy the physiological activity of the ACTH.

C. Defects in Example 9c by Kurjan et al.

(1) The problems introduced by blunt-end ligation of the fragments would be even more severe in this example, since at least three fragments are being ligated making the formation of improperly oriented DNA ligations the far majority. There are no measures described which ensure proper orientation of the fragments or which allow release of the desired ligation product from linear ligation products to

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enable cloning into the appropriate plasmid vector since the EcoRI site has been permanently destroyed by filling in enzymatically.

(2) Again, there is no translational stop codon introduced into the 3' fragment. Therefore, translational read-though will lead to the production of a fusion protein with an unknown C-terminal extension. Such extensions are known to interfere with proper secretion of the fusion protein and also change the character of the product produced from an enkephaline to a fusion peptide product containing an amino acid sequence of unknown length and composition which is not an enkephalin.

I, Ronald A. Hitzeman, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/2/04
Date

Ronald A. Hitzeman
RAH

RESUME AND CURRICULUM VITAE

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The initial focus of Genotypes, Inc. is to work for other companies to help them solve specific product production problems on a contractual basis. Examples of problems to solve have been yield improvement of specific gene products (proteins for pharmaceutical applications) by production strain (microorganisms) changes as well as product quality improvement by modification of transcriptional, translational, or posttranslational pathways. More complex alterations or additions to biochemical pathways in yeast have been performed. The microorganisms focused on are mainly the yeast, *Saccharomyces cerevisiae*, (some familiarity with the yeast, *Pichia pastoris*) and various bacteria. The word, genotypes, refers to the genetic pedigree or characteristics of a living organism. Our experimentation involves making changes at the DNA level (in chromosomes and plasmids) using a combination of biochemistry, molecular biology, and genetics to manipulate and improve production microorganisms for pharmaceutical and industrial applications. Improved protein production strains of *Saccharomyces cerevisiae* and improved expression plasmids are available from Genotypes for licensing.

PERSONAL

Date of Birth:	May 2, 1948
Citizenship:	United States
Marital Status:	Married; with 29 year-old son, M.D. from UCLA, resident at Sutter General Hospital in Sacramento.
Present Position:	President and Research Director of Genotypes, Inc., a consulting company located in the Bay Area (incorporated March 6th, 1992).

EDUCATION

Institution	Dates	Degree
Purdue University	9/66 - 6/70	B.S., Chemistry
The University of Michigan	9/71 - 5/73	M.S., Biochemistry
The University of Michigan	5/73 - 6/77	Ph.D., Biochemistry Supervisor: Dr. Alan R. Price
The University of California at Santa Barbara	7/77 - 5/80	Postdoctoral fellow Supervisor:Dr. John Carbon

ACADEMIC HONORS

Dean's List at Purdue University, 1966-1970
Academic Award for graduating within the top 10% at Purdue University, 1970

APPOINTMENTS

Teaching Fellow, Department of Biochemistry, The University of Michigan, 1971-1975, USPHS, NIH Training Grant 5 T01 GM00187-17.

Teaching Assignments:
Biochemistry for Nursing Students - fall 1972
Biochemistry for Dental Students - winter 1972
Biochemistry for Undergraduate and Graduate non-majors - fall 1973
Biochemistry Undergraduate Course - fall 1974

Graduate Student Research Assistant, The University of Michigan, 1975-1977, ERDA, Grant EY-76-S-02-2101.

Damon Runyon-Walter Winchell postdoctoral fellow in the laboratory of Dr. John Carbon at The University of California, 1977-1979.

Abbott Laboratories postdoctoral fellow in the laboratory of Dr. John Carbon at The University of California, August 1, 1979-May 31, 1980.

WORK EXPERIENCE:

2000-present

Consultant (as President of Genotypes) for separate companies helping with strain development, product development and improvement, and yield improvement:

1) Sequoia Biotech – two GenenExers involved with probiotics development:
   Frank Hagie and Dr. Glen Nedwin – Genotypes will run a lab to develop yeast and bacteria for probiotic delivery of protein products.
2) YPS (Yeast Protein Sciences)(for about 1 year) – production of pharmaceutical proteins in yeast, esp. human antibodies – Robert Leach headed this company – I have done some lab direction and bench work at Penn State University with a collaborator, Dr. Davis Ng. Company shut down 12/03 due to intellectual property problems.
3) Nastech – I have worked as a consultant for years and research reviewer in April, 2003. The Company is developing a platform technology for delivering both new small- and large molecular drugs by nasal administration. CEO is Dr. Steven Quay.
4) Patent work for Genentech
5) Genotypes solely owns US patent # 6,670,154 B1 (listed as #10 below, described reference 38) which describes the making of photosynthetic yeast for alcohol production. I am currently looking for someone to finance this work as well as to work on photosynthetic organisms to make alternative fuels.

March, 1992-2000

President of Genotypes, Inc., had a company lab located at 61 Airport Blvd. (Suite B) in South San Francisco with its mission to supply research lab work as well as consulting advice to client biotech companies. This was a natural expansion of the work I have been doing for many years. Some of the clients I have worked with in the past supported this effort as well as new clients. See 1st page for more extensive mission. Had up to 9 people under my supervision.

Jul.,1991-Oct., 1992

Consultant and researcher for Strohtech, a division of Stroh Brewery. Also worked for Strohtech (now Apex Bioscience, Inc.) through Genotypes.

Jan.,1991-Sept.,1992

Consultant and directed researchers for Amylin in San Diego- peptide product yield improvement and product modification enhancement.

July-Dec.,1990

Consultant for Phage (Pharmacia Genetic Engineering) and Kabi in San Diego. Ended due to shut down of facility by its owner company, Procordia, of Sweden. I was doing 1/2 time research at this facility and directing others.

Jan., 1990-Jan.,1993

Visiting Scientist (part-time) at University of California, Berkeley, Department of Biochemistry, in lab of Dr. Clinton Ballou. Further study of yeast secretion/glycosylation mutants that were isolated at Genentech as well as many new mutant strains isolated at Berkeley.

Oct., 1989-Oct., 1993

Consultant for many other companies.

October, 1989- Dec. 1990

Consultant, Kabi Peptide Hormones. Worked at Kabi (Stockholm,Sweden) - November and December, 1989 – EGF and IGF-1 peptide yield improvement from yeast.

October, 1989- present

Consultant for Genentech. Patent work ongoing.

June, 1980- Sept., 1989

Research Scientist at Genentech, Inc. (see publications 12-33, 36, and all patents except 4 and 8-11)

Dec., 1982- Sept., 1989

Senior Scientist, Department of Cell Genetics
Genentech, Inc.
460 Point San Bruno Boulevard
South San Francisco, CA

June,1981 - Dec., 1982
Scientist I, Department of Molecular Biology,
Genentech, Inc.

June 1980-June 1981
Scientist II, Department of Molecular Biology,
Genentech, Inc.

Management Experience:

I. I have managed from 2 to 9 people during my tenure as yeast lab director at Genentech (1980-Sept. 1989). Those reporting to me ranged from B.S. through Ph.D. degrees (postdoctoral fellows and more senior Ph.D.'s). I also was yeast project team leader for many years managing many different projects.

II. At the same time at Genentech, I also directed a media preparation laboratory for about 3 years with 3-4 people.

III. I have performed and managed research at Genotypes, managing from 4-9 people from 1992-2000. I also have directed the company as president and visited other companies to negotiate research contracts as well as to consult.

Some of My Research Collaborators:

1992- present	Partial list of clients (some confidential): Pfizer, Inc., Cephalon, Apex Bioscience, Khepri, Collagen Corp., Cohesion Technologies, Icos Corporation, Corvas International, Canji, Nastech, YPS, and Sequoia Biotech.	
1990 – 1993	Isolation and characterization of yeast mutants that affect glycosylation and golgi transport of secreted proteins Barker Hall.	Dr. Clinton Ballou Dept. of Biochemistry Barker Hall U.C. Berkeley
1988 - 1990	Production and Function of Mammalian Na^+/K^+ ATPase in Yeast	Dr. Robert Farley University of Southern California, School of Medicine
1987 - 1990	Growth Factor Secretion by Yeast	Kabi Peptide Hormones Dr. Par Gellerfors, Sweden
1983 - 1989	Human Serum Albumin Secretion by Yeast	Mitsubishi, Corp.
1985 - 1986	Human and Yeast Chimeric Phosphoglycerate Kinases (Gene and Protein Function)	Drs. Arthur Riggs and Maria Mas City of Hope, Los Angeles
1980 - 1981	Heterologous Gene Expression in Yeast	Drs. Benjamin Hall and Gustav Ammerer University of Washington

REFERENCES:

Dr. Richard A. Berg Vice President of Research and Development	Previously with Collagen Corp., and Cohesion Technologies. Now with FzioMed, Inc. 170-A Granada Drive San Luis Obispo, CA 93401 Phone: 805-546-0610 Fax: 805-546-0571 raberg@fzio.com
Dr. John Carbon (Postdoctoral Director) Professor Biological Sciences	University of California Department of Biological Sciences Santa Barbara, CA 93106
Dr. Maurille J. Fournier Professor Biological Chemistry	University of Massachusetts Department of Biochemistry Amherst, MA 01003
Dr. Dennis Henner Staff Scientist Vice President of Research	Was at Genentech, Inc 460 Point San Bruno Blvd. South San Francisco, CA 94080 Now at MPM Capital, home:650-359-2199
Mr. Joseph De Angelo Vice President of Research Apex Bioscience, Inc.	P.O. Box 12847 Research Triangle Park North Carolina 27709-2847 Phone: 919-405-4002 Email: jdeangelo@apexbioscience.com
Dr. Pat Gray Senior Director of Science	Was with ICOS Corporation 22021 20th Ave. S.E. Bothell, Washington 98021 Phone: 206-322-2286 206-650-6765

Ronald Hitzeman, Ph.D.

President and CEO of Genotypes, Inc. - founded Genotypes in 1992. Previously, Dr. Hitzeman was a senior scientist at Genentech, Inc. for approximately nine years during which time he was the first scientist (in collaboration with scientists from the University of Washington) to successfully express a heterologous protein, Leukocyte Interferon, in yeast. This led to a major patent, "Expression of Polypeptides in Yeasts". Dr. Hitzeman received his doctorate in Biochemistry from The University of Michigan in 1977 and was a postoctoral fellow from 1977-1980 at UC Santa Barbara under the supervision of Dr. John Carbon, a leading yeast researcher in Molecular Biology. Dr. Hitzeman's list of publications and patents are as follows:

PUBLICATIONS

1. Price, A.R., R. Hitzeman, J. Frato, and K. Lombardi. 1974. Rifampicin-Resistant Bacteriophage PBS2 Infection and RNA Polymerase in *Bacillus subtilis*. *Nucleic Acid Res.* 1: 1497-1502.

2. Hitzeman, R.A. 1978. DNA Polymerase Induced by *Bacillus subtilis* Bacteriophage PBS2. Ph.D., dissertation. University of Michigan.

3. Hitzeman, R.A., A.R. Price, J. Neuhard, and H. Mollgaard. 1978. Deoxyribonucleoside Triphosphates and DNA Polymerase in Bacteriophage PBS1-Infected *Bacillus subtilis*. In *DNA Synthesis; Present and Future* (Molineaux, I.J., and Kohiyama, M., eds.) Plenum Press, New York. pp. 255-266.

4. Hitzeman, R.A., A.N., Hanel, and A.R. Price. 1978. Dextran Sulfate as a Contaminant of DNA extracted from Concentrated Viruses and as an Inhibitor of DNA Polymerases. *J. Virol.* 27: 255-257.

5. Hitzeman, R.A., and A.R. Price. 1978. *Bacillus subtilis* Bacteriophage PBS2-Induced DNA Polymerase; Its Purification and Assay Characteristics. *J. Biol. Chem* 253: 8518-8525.

6. Hitzeman, R.A., and A.R. Price. 1978. Characterization of *Bacillus subtilis* Bacteriophage PBS2-Induced DNA Polymerase and Its Associated Exonuclease Activity. *J. Biol. Chem.* 253: 8526-8532.

7. Hitzeman, R.A. and A.R. Price. 1978. Relationship of *Bacillus subtilis* DNA Polymerase III to Bacteriophage PBS2-Induced DNA Polymerase and to the Replication of Uracil-Containing DNA. *J. Virol.* 28: 697-709.

8. Clarke, L., R. Hitzeman, and J. Carbon. 1979. Selection of Specific Clones from Colony Banks by Screening with Radioactive Antibody. *Methods Enzymol.*, 68: 436-442.

9. Hitzeman, R.A., A.C. Chinault, A.H. Kingsman, and J. Carbon. 1979. Detection of E. coli Clones Containing Specific Yeast Genes by Immunological Screening. In volume 14 of the series, ICN-UCLA Symposium on Molecular and Cellular Biology (Academic Press) pp. 57-68.

10. Hitzeman, R.A., L. Clarke, and J. Carbon. 1980. Isolation and Characterization of the Yeast 3-Phosphoglycerokinase Gene (PGK) by an Immunological Screening Technique. *J. Biol. Chem.* 255: 12073-12080.

11. Chalmers, J., M. Kuziora, R. Hitzeman, J. Carbon, and S. Wakil. 1983. Molecular Cloning of Fatty Acid Synthetase Genes from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 258: 11648-11653.

12. Hitzeman, R.A., F.E. Hagie, H.L. Levine, D.V. Goeddel, G. Ammerer and B.D. Hall. 1981. Expression of Human Gene for Interferon in Yeast. *Nature* 293: 717-722.

13. Ammerer, G., R. Hitzeman, F. Hagie, A. Barta, and B.D. Hall. 1981. The Functional Expression of Mammalian Genes in Yeast. In *Recombinant DNA, Proceedings of the Third Cleveland symposium on Macromolecules* (Walton, A.G., ed) Elsevier Scientific Publishing Company, Amsterdam, pp. 185-197.

14. Hitzeman, R.A., F.E. Hagie, J.S. Hayflick, C.Y. Chen, P.H. Seeburg and R. Derynck. 1982. The Primary Structure of the *Saccharomyces cerevisiae* Gene for 3-Phosphoglycerate Kinase. *Nucleic Acids Res.* 10: 7791-7808.

15. Hitzeman, R.A., C.Y. Chen, F.E. Hagie, E.J. Patzer, C.C. Liu, D.A. Estell, J.B. Miller, A. Jaffe, D.G. Kleid and A.D.Levinson, and H. Oppermann. 1983. Expression of Hepatitis B Virus Surface Antigen in Yeast. *Nucleic Acids Res.* 11: 2745-2763

16. Derynck, R., P.W. Gray, E. Yelverton, D.W. Leung, H.M. Shepard, R.M. Lawn, A. Ullrich, R. Najarian, D. Pennica, F.E. Hagie, R.A. Hitzeman, P.J. Sherwood, A.D. Levinson, and D.Y. Goeddel. 1982. Synthesis of human Interferons and Analogs in heterologous Cells, ICN-UCLA Symposium, Volume, pp. 249-262.

17. Derynck, R., R.A. Hitzeman, P.W. Gray and D.V. Goeddel. 1983. Expression of Human Interferon-a in Heterologous Systems. In *Experimental Manipulation of Gene Expression* (Inouye, M., Ed) Academic Press, N.Y., N.Y., pp. 247-258.

18. Hitzeman, R.A., D.W. Leung, L.J. Perry, W.J. Kohr, F.E. Hagie, C.Y. Chen, J.M. Lugovoy, A. Singh, H.L. Levine, R. Wetzel, and D.V. Goeddel. 1982. Expression, Processing and Secretion of Heterologous Gene Products by Yeast. In *Proceedings of the Berkeley Workshop on Recent Advances in Yeast Molecular Biology: Recombinant DNA*, vol., 1, University of California, Berkeley, pp. 173-190.

19. Hitzeman, R.A., D.W. Leung, L.J. Perry, W.J. Kohr, H.L. Levine, and D.V. Goeddel. 1983. Secretion of Human Interferons by Yeast. *Science* 219: 620-625.

20. Singh, A., E.Y. Chen, J.M. Lugovoy, C.N. Chang, R.A. Hitzeman, and P.H. Seeburg. 1983. *Saccharomyces cerevisiae* Contains Two Discrete Genes Coding for the □-Factor Pheromone. *Nucleic Acids Res.* 11: 4049-4063.

21. Hitzeman, R.A., C.Y. Chen, F.E. Hagie, J.M. Lugovoy, A. Singh. 1984. (Review) Yeast: An Alternative Organism for Foreign Protein Production. In *Recombinant DNA Products: Insulin-Interferon-Growth Hormone* (Bollon, A.P., ed.) CRC Press, Boca Raton, Florida, pp. 47-65.

22. Chen, C.Y., H. Opperman, and R.A. Hitzeman. 1984. Homologous Versus Heterologous Gene Expression in the Yeast, *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 23: 8951-8970.

23. Hitzeman, R.A., C.N. Chang, M. Matteucci, J. Wulf, J.M. Lugovoy, C.Y. Chen, L.J. Perry, W.J. Kohr, and A. Singh. 1986. Construction of Expression Vectors for Secretion of Human Interferons by Yeast. *Methods Enzymol.* 119: 424-433.

24. Chang, C.N., M. Matteucci, L.J. Perry, J.J. Wulf, C.Y. Chen, and R.A. Hitzeman. 1986. Secretion of Human Leukocyte Interferon Using the Yeast Invertase Signal Sequence. *Molecular and Cellular Biology* 6: 1812-1819.

25. Singh, A., C.N. Chang, J.M. Lugovoy, M.D. Matteucci, and R.A. Hitzeman. 1983. Strategies for the Production of Pharmaceutical Proteins in Microorganisms. In *Genetics: New Frontiers* (Oxford and IBH Publishing Co.) pp. 169-177.

26. Hitzeman, R.A., D.W. Leung, L.J. Perry, W.J. Kohr, H.L. Levine, and D.V. Goeddel. 1984. Secretion of Human Interferons by Yeast. In *Biotechnology and Biological Frontiers*, Ed. P.H. Abelson (AAAS) pp. 21-32.

27. Etcheverry, T., W. Forrester, and R. Hitzeman. 1986. Regulation of the Chelatin Promoter during the Expression of Human Serum Albumin or Yeast Phosphoglycerate Kinase in Yeast. *Biotechnology* 4: 726-730.

28. Mas, M.T., C.Y. Chen, R.A. Hitzeman, and A.D. Riggs. 1986. Active human yeast chimeric phosphoglycerate kinases engineered by domain interchange. *Science* 233: 788-790.

29. Chen, C.Y. and R.A. Hitzeman. 1987. Human, Yeast and Hybrid 3-Phosphoglycerate Kinase Gene Expression in Yeast. *Nucleic Acids Research* 15: 643-660.

30. Chen, C.Y. and R.A. Hitzeman. 1987. Possible Translational Control Mechanisms Associated with Gene Expression in Yeast. *Biological Research on Industrial Yeasts*. CRC Press, Ed. Steward, Russell Klein, and Hiebsch, pp. 71-86.

31. Chisholm, V., C.Y. Chen, N.J. Simpson, and R.A. Hitzeman. 1990. A Molecular and Genetic Approach to Enhancing Protein Secretion. In *Gene Expression Technology . Methods of Enzymology* 185: 471-482.

32. Hitzeman, R.A., C.Y. Chen, D.J. Dowbenko, M.E. Renz, A. Singh, V. Chisholm, R. Hamilton, and C.N. Chang. 1990. The Use of Heterologous and Homologous Signal Sequences for the Secretion of Heterologous Proteins from Yeast. In *Gene Expression Technology . Methods of Enzymology* 185 : 421-440.

33. Horowitz, B., Eakle, K.A., Scheiner-Bobis, G., Randolph, G.R., Chen, C.Y., Hitzeman, R.A., and Farley, R.A. 1990. Synthesis and Assembly of Functional Mammalian Na,K-ATPase in Yeast. *J. Biol. Chem.* 265 : 4189-4192.

34. Ballou, L., R. A. Hitzeman, M. S. Lewis and C. E. Ballou. 1991. Vanadate-Resistant Yeast Mutants are Defective in Protein Glycosylation.*Proc. Nat. Acad. Sci. USA.* 88: 3209-3212.

35. Berka, R. M., F.T. Bayliss, P. Bloebaum, D. Cullen, N. S. Dunn-Coleman, K. H. Kodama, K.J. Hayenga, R. A. Hitzeman, M. H. Lamsa, M. M. Przetak, M. W. Rey, L. J. Wilson, and M. Ward. 1992. *Aspergillus niger* var. *awamori* as Host for the Expression of Heterologous Genes. In *Applications in Enzyme Biotechnology*. Plenum Press.

36. Toman, P. D., Chisholm, G., McMullin, H., Giere, L. M., Olsen, D. R., Kovach, R. J., Leigh, S. D., Fong, B.E., Chang, R., Daniels, G. A., Berg, R. A., and Hitzeman, R. A. 2000. Production of Recombinant Human Type I Procollagen Trimers Using a Four-gene Expression System in the Yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 275: 23303-23309.

37. Olsen, D. R., Leigh, S. D., Chang, R., McMullin, H., Ong, W., Tai, E., Chisholm, G., Birk, D. E., Berg, R. A., Hitzeman, R. A., and Toman, P. D. 2001. Production of Human Type I Collagen in Yeast Reveals Unexpected New Insights into the Molecular Assembly of Collagen Trimers. *J. Biol. Chem.* 276: 24038-24043.

38. Chisholm, G., Giere, L. M., Weaver, C. I., Loh, C. Y., Fong, B. E., Bowser, M. E., Hitzeman, N. C., and Hitzeman, R. A. 2002. Automatic Eukaryotic Artificial Chromosomes: Possible Creation of Bacterial Organelles in Yeast. In *Horizontal Gene Transfer*, Chapter 22, Academic Press, NY and London, 2nd Edition, Eds. Michael Syvanen and Clarence Kado, pp 249-259.

PATENTS (many filings and patents in other countries not shown):

1. United States Patent #4,803,164, February 7, 1989. Submitted August 31, 1981. Preparation of Hepatitis B Surface Antigen in Yeast. Inventors: R.A. Hitzeman, A.D. Levinson, and D.G. Yansura.
Assignee: Genentech, Inc. (foreign patent documents also).

2. United States Patent #4,775,622, October 4, 1988. Submitted March 8, 1982. Expression, Processing, and Secretion of Heterologous Protein by Yeast. Inventors: R.A. Hitzeman and D.W. Leung. Assignee: Genentech, Inc. (foreign patent documents also).

3. Expression of Polypeptides in Yeast. Priority February 25, 1981. Inventors: R.A. Hitzeman, F.E. Hagie, B.D. Hall, and G. Ammerer. Applicants: Genentech, Inc. and the Board of Regents of the University of Washington. Patents are administered by WRF (Washington Research Foundation, http://www.wrfseattle.org/). US Patent Nos. 5,618,676, 5,854,018 5,856,123, and 5,919,651.

4. Novel Eukaryotic Vectors and Plasmids Having PGK Regulatory Signals. U.S. Patent Application Filed November 25, 1981.
Inventors: R.A. Hitzeman and J. Carbon. Assignee: U.C. Regents, a California Corporation (U.C. Case: 81-201-1). U.S. patent granted the end of 1989. US patent # 4,865,989.

5. Metallothionein Transcription Control Sequences and Use Thereof. Filing date: May 17, 1984. Inventors: Tina Etcheverry and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #100-244). US patent # 4,940,661 July 10, 1990.

6. Use of Yeast Homologous Signals to Secrete Heterologous Proteins. Filing date: April 25, 1983. Inventors: Chung Nan Chung, Mark Matteucci, and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #100-129). Other Patents (submitted for approval), United States Patent #5,010,003, April 23, 1991 (foreign patents also).

7. Yeast Strains for Increased Production and Secretion of Heterologous Proteins. Filing date: April 26, 1989.
Inventors: Robert Hamilton, Vanessa Chisholm, and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #6464).

8. Production of Recombinant Procollagen in Yeast. U.S. Patent Application Serial No. 08/546,047, filing date, 10/20/95. International patent application no. PCT/US96/16646, filing date 10/18/96. Inventors: D. Toman, R. Berg, G. Daniels, R. Hitzeman, and G. Chisholm, IV. Assignees: Collagen Corporation and Genotypes, Inc. WO97/14431. **Now US patent #6,472,171 on October 29, 2002.**

9. Novel Methods for the Production of Gelatin and Full-Length Triple Helical Collagen in Recombinant Cells. U.S. Patent Application Serial No. 60/084,828, filed May 8, 1998. (RECENTLY GRANTED, U.S. patent # 6,413,742, July 2, 2002 and Australian patent # 754608)
Inventors: R. Hitzeman, G. Chisholm, R. Chang, H. McMullin, and D. Olsen.
Assignees: Cohesion Technologies and Genotypes, Inc.

10. Novel Vectors and Methods for Transfer of Bacterial Genomes and Other DNAs into Eukaryotic Organisms to Add New Valuable Functions to the Eukaryotes. U.S. Patent Serial Application No. 60/094,294, filing date. 7/27/98.
Inventors: R. Hitzeman and G. Chisholm, IV. Assignee: Genotypes, Inc. US patent granted May 2003 – received notice of allowance. US patent # 6,670,154 B1, December 30, 2003.